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REPLACEMENT APPEAL BRIEF Address to: Mail Stop Appeal Brief-Patents Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Application Number	09/293,670
	Confirmation Number	5176
	Attorney Docket No.	RIGL-036CIP
	Filing Date	April 16, 1999
	First Named Inventor	Joseph Fisher
	Examiner	Teresa D. Wessendorf
	Group Art	1639
	Title: *Multiparameter FACS Assays to Detect Alterations in Cellular Paramters and to Screen Small Molecule Libraries*	

Sir:

This Appeal Brief replaces the Appeal Brief filed on January 28, 2008

This Brief is filed in support of Appellants' appeal from the Examiner's Rejection dated August 10, 2007. No claims have been allowed. Claims 17-36 are pending. Claims 17-26, 30 and 32 are appealed. A Notice of Appeal was filed on November 13, 2007.

The Board of Appeals and Interferences has jurisdiction over this appeal pursuant to 35 U.S.C. §134.

It is believed that no fees are due. If, however, the PTO finds that for some reason a fee is due, the Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 which may be required by this paper, to deposit account number 50-0815, reference no. RIGL-036 CIP.

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REAL PARTY IN INTEREST

The inventors named on this patent application assigned their entire rights to the invention to Rigel Pharmaceuticals, Inc.

RELATED APPEALS AND INTERFERENCES

There are currently no other appeals or interferences known to Appellants, the undersigned Appellants' representative, or the assignee to whom the inventors assigned their rights in the instant case, which would directly affect or be directly affected by, or have a bearing on the Board's decision in the instant appeal.

STATUS OF CLAIMS

Claims 1-16 were canceled. Claims 17-36 are pending. During the course of prosecution, claims 26-29, 31, 33-36 were withdrawn by the Examiner. Claims 17-26, 30 and 32 are rejected and are appealed herein.

STATUS OF AMENDMENTS

No amendments to the claims were filed subsequent to issuance of the prior Office Action.

SUMMARY OF CLAIMED SUBJECT MATTER

The claimed invention is drawn to a method for screening for an alteration in cellular phenotype. The method includes providing a population of cells comprising a library of retroviral vectors encoding different candidate bioactive agents; sorting the population of cells based on at least five parameters using fluorescence activated cell sorting (FACS); and detecting at least one cell of the population having the alteration in the cellular phenotype. The cellular phenotype is selected from a group of cellular

phenotypes consiting of cell cycle, apoptosis, exocytosis, expression of a cell surface receptor, and expression of a receptor protein.

Below is a description of the independent claim and where support for can be found in the specification.

Independent Claim 17 claims a method of screening for an alteration in cellular phenotype (page 3, line 37 – page 4, line 1). The method includes: a) providing a population of cells comprising a library of retroviral vectors encoding different candidate bioactive agents (page 6, lines 1-11, Fig. 1, and page 19, lines 8-37); b) sorting the population of cells based on at least five parameters using fluorescence activated cell sorting (FACS) (page 4, lines 1-4; and c) detecting at least one cell of the population having the alteration in cellular phenotype (page 15, line 37 – page 16, line 1). The cellular phenotype is selected from a group of cellular phenotypes consisting of cell cycle (page 2, line 11-24), apoptosis (page 11, lines 11-17), exocytosis (page 2, line 26 – page 3, line 33), expression of a cell surface receptor (page 8, line 13), and expression of a receptor protein (page 8, lines 20-24).

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

I. Rejection of claims 17-24 and 30 under 35 U.S.C. § 103(a) over Uhr et al. (US 5612185) in view of Conneally et al. (*Blood* 1996, 87: 456-464).

II. Rejection of claims 17-25, 30, and 32 under 35 U.S.C. § 103(a) over Nolan (WO 97/27212), in view of Jia-ping (*Chinese Journal of Physical Medicine* 1995, 17:168-171) and Uhr et al.

III. Rejection of claim 26 under 35 U.S.C. § 103(a) over Nolan, in view of Jia-ping, Uhr et al., Hide et al. (*J. Cell Bio.* 1993, 123:585-593) , and the Appellants' disclosure.

ARGUMENT

I. Claims 17-24 and 30 stand rejected under 35 U.S.C. § 103(a) as being obvious over Uhr et al. in view of Conneally et al.

Claims 17-24 and 30 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Uhr et al. (US 5612185) in view of Conneally et al. (*Blood* 1996 87: 456-464). As best understood by the Appellants, the Examiner believes that Uhr's method for identification of tumor cell types, together with Conneally's teaching of retroviral-mediated gene transfer, renders the claims obvious.

The following arguments are directed to all claims. For the purposes of this appeal, all claims stand or fall together. Claim 17 is representative and set forth below.

> 17. A method of screening for an alteration in cellular phenotype, said method comprising:
> a) providing a population of cells comprising a library of retroviral vectors encoding different candidate bioactive agents;
> b) sorting said population of cells based on at least five parameters using fluorescence activated cell sorting (FACS); and
> c) detecting at least one cell of said population having said alteration in said cellular phenotype;
> wherein said cellular phenotype is selected from a group of cellular phenotypes consisting of cell cycle, apoptosis, exocytosis, expression of a cell surface receptor, and expression of a receptor protein.

In a nutshell, the Appellants submit that the claims are not obvious in view of the cited references because neither of the cited references provide a library of retroviral vectors.

As best understood by the Appellants, the Examiner believes that Uhr's method for identifying tumor cell types, in combination with Conneally's method of retroviral-mediated gene transfer, renders the claims obvious.

In order to meet its burden in establishing a rejection under 35 U.S.C. § 103 the Office must first demonstrate that the combined prior art references teach or suggest all the claimed limitations, so as to present

> A finding that the prior art included each element claimed [...] with the only

> difference between the claimed invention and the prior art being the lack of actual combination…[1]

It is also well established that rejections based on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning to demonstrate that a person of ordinary skill in the art would have been prompted to combine elements in the way a claimed invention does. See also e.g., KSR Int'l Co. v. Teleflex Inc., 127 S. Ct. 1727, 1740 (2007):

> "[A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art."[2]

As set forth in the arguments below, the Appellants contend that all cited references are deficient for not teaching or suggesting a method that involves cells comprising a library of retroviral vectors, as required by the rejected claims.

In maintaining this rejection, the Examiner points towards Uhr's column 22, lines 14-20, Fig. 3, and Example 2 and argues that those sections teach a library of retroviral vectors encoding different candidate bioactive agents. However, a detailed analysis of these sections reveals that Uhr does not teach or suggest a population of cells comprising a library of retroviral vectors. When read in context, Uhr, in column 22, teaches that tumor cell cycle arrest may be induced by gene therapy and that a retrovirus may be used to introduce gene constructs. Likewise, Uhr's Fig.3 and Example 2 relate to the expression of oncogenes in tumor cells by assessing mRNA levels of c-myc and c-fos. Hence, these passages are unrelated to cells comprising a library of retroviral vectors.

At no point in Uhr's disclosure, including passages relied upon by the Examiner, does Uhr teach or suggest a library of retroviral vectors.

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1 Federal Register vol. 72, No. 195, Oct 10, 2007. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)

2 See also Pharmastem Therapeutics v. Viacell et al., 2007 U.S. App. LEXIS 16245 (Fed. Cir. 2007);Omegaflex, Inc. v. Parker-Hannifin Corp., 2007 U.S. App. LEXIS 14308 (Fed. Cir. 2007) Dystar Textilfarben GmbH v. C.H. Patrick Co., 464 F.3d 1356, 1360 (Fed. Cir. 2006) In re Kahn, 441 F.3d 977,985 (Fed. Cir. 2006). Medichem, 437 F.3d at 1164. *In re Fulton*, 391 F.3d 1195, 1199-1200 (Fed. Cir. 2004)

In attempting to fill the void between Uhr's disclosure and the rejected claims, the Examiner cites Conneally and asserts the Conneally's teaching of retroviral-mediated gene transfer renders the rejected claims obvious. However, Conneally, like Uhr, fails to provide a library of retroviral vectors. Specifically, in the discussion section cited by the Examiner, Conneally teaches that having a cell surface marker such as CD24 encoded by retroviral constructs can facilitate identification and selection of cells. This passage does not provide a library of retroviral vectors, as recited in claim 17.

For each of the reasons set forth above, Uhr alone or in combination with Conneally does not teach or suggest each and every element of the rejected claims. Since Claim 17 is the only independent claim of this application, the arguments presented above apply with equal force all other rejected claims. Therefore, the Appellants respectfully request the reversal of 103(a) rejections of claims 17-24 and 30 on this basis.

II. Claims 17-25, 30, and 32 are rejected under 35 U.S.C. § 103(a) as being obvious over Nolan, in view of Jia-ping and Uhr et al.

Claims 17-25, 30, and 32 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Nolan et al. (WO 97/27212) in view of Jia et al. (*Chinese Journal of Physical Medicine*), and in further view of Uhr et al. The following arguments are directed to all claims.

The Appellants submit that Nolan cannot preclude the patentability of the rejected claims for the reasons set forth below.

This instant application's earliest priority date is April 17, 1998, as indicated on the filing receipt and the application data sheet of this application. The relevant section of the filing receipt is reproduced below for the Board's convenience.

Domestic Priority data as claimed by applicant

This application is a CIP of 09/157,748 09/21/1998 PAT 6,461,813
which is a CIP of 09/062,330 04/17/1998 PAT 6,897,031 .

Thus, the instant application claims priority to an application (09/062,330) that was filed on *April 17, 1998*.

Nolan's publication date (July 31, 1997) predates the earliest priority date of this application (April 17, 1997) by less than a year. As such, Nolan only qualifies as prior art only under 35 U.S.C. § 102(a)[3].

A Declaration under 35 U.S.C. § 1.131 (the "Fisher Declaration"; submitted herein in the Evidence Appendix of this brief) was submitted with the Appellants' response dated July 24, 2006, in order to obviate a rejection over a similar combination of references (i.e., Nolan in view of Jai-ping or Ryan). The Fisher Declaration establishes invention of the subject matter of the rejected claims prior to the Nolan's publication date and, as such, Nolan cannot preclude the patentability of the instant claims.

In maintaining this rejection, the Examiner neither discusses the contents of the Fisher Declaration nor provides any evidence that the Applicants did not antedate Nolan's publication date. Rather, the Examiner counters the Appellants' position by simply stating that:

> "Nolan reference was published more than one year of applicants' earliest filing date. Thus, the 35 U.S.C. § 1.131 declaration does not overcome the 103 rejection...."

See page 13 of the Office Action dated August 10, 2007.

As noted above, however, the filing receipt itself states that this application claims priority to an application that was filed on *April 17, 1998.* Since Nolan was published on July 31, 1997, Nolan was published *less than* one year before the Appellants' earliest priority date. As such, the Examiner's position, i.e., that "Nolan was published more than one year of applicants' earliest filing date" lacks support.

In view of the foregoing discussion, the Applicants submit that Nolan is

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[3] The PCT application upon which Nolan's publication (WO97/27212) is based was filed on January 23, 1997. Nolan's filing date is *prior to* the November 19, 2000 date of enactment of amended 35 U.S.C. § 102(e). As such, Nolan is not available as prior art as of its filing date, and

disqualified as a prior art reference and cannot preclude the patentability of the instant claims. Thus, this rejection should be reversed.

III. Claims 26 stands rejected under 35 U.S.C. § 103(a) as being obvious over Nolan, in view of Jia-ping, Uhr et al., Hide et al., and the Appellants' disclosure.

Claim 26 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Nolan et al., in view of Jia et al., Uhr et al. and the Appellants' disclosure. The following arguments are directed to all claims.

As noted in the previous section, the Appellants submit that Nolan cannot be used as prior art under 35 U.S.C. § 103(a) because the subject invention predates the publication date of Nolan.

As such, the Appellants submit that Nolan is disqualified as a prior art reference and cannot preclude the patentability of the instant claims. The Appellants respectfully request the reversal of this rejection.

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is not citable as "102(e)-type art".

SUMMARY

I. Claims 17-24 and 30 are not obvious under 35 U.S.C. § 103(a) over Uhr et al. (US 5612185) in view of Conneally et al.

II. Claims 17-25, 30, and 32 are not obvious 35 U.S.C. § 103(a) over Nolan (WO 97/27212), in view of Jia-ping and Uhr et al.

III. Claims 26 is not obvious under 35 U.S.C. § 103(a) over Nolan, in view of Jia-ping, Uhr et al., Hide et al., and the Appellants' disclosure.

RELIEF REQUESTED

The Appellants respectfully request that the rejections of Claims 17-26, 30, and 32 under 35 U.S.C. § 103(a) be reversed, and that the application be remanded to the Examiner with instructions to issue a Notice of Allowance.

Respectfully submitted,

Date: May 7, 2008

By: /James S. Keddie, Reg. No. 48,920/
James S. Keddie, Ph.D.
Registration No. 48,920

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CLAIMS APPENDIX

1-16. (Cancelled)

17. (Previously presented) A method of screening for an alteration in cellular phenotype, said method comprising:

a) providing a population of cells comprising a library of retroviral vectors encoding different candidate bioactive agents;

b) sorting said population of cells based on at least five parameters using fluorescence activated cell sorting (FACS); and

c) detecting at least one cell of said population having said alteration in said cellular phenotype;

wherein said cellular phenotype is selected from a group of cellular phenotypes consisting of cell cycle, apoptosis, exocytosis, expression of a cell surface receptor, and expression of a receptor protein.

18. (Previously presented) The method according to Claim 17, wherein said candidate agent comprises a fusion partner.

19. (Previously presented) The method according to Claim 18, wherein said fusion partner is a fluorescent protein.

20. (Previously presented) The method according to Claim 19, wherein said fluorescent protein is a green fluorescent protein (GFP).

21. (Previously presented) The method of Claim 17, wherein the cell is a mammalian cell.

22. (Previously presented) The method of Claim 21, wherein said mammalian cell is a tumor cell.

23. (Previously presented) The method of Claim 21, wherein said mammalian cell is a human cell.

24. (Previously presented) The method of Claim 23, wherein said human cell is a human tumor cell.

25. (Previously presented) The method of Claim 17, wherein said cellular phenotype is exocytosis.

26. (Previously presented) The method of Claim 25, wherein said sorting of said population of cells using fluorescence activated cell sorting (FACS) is based on at least five parameters selected from the group consisting of: light scattering, fluorescent dye update, fluorescent dye release, annexin granule binding, surface granule enzyme

activity, and the quantity of granule specific proteins.

27. (Withdrawn) The method of Claim 26, wherein at least one of said five parameters is fluorescent dye uptake and wherein said fluorescent dye is a styryl dye.

28. (Withdrawn) The method of Claim 26, wherein at least one of said five parameters is surface granule enzyme activity and wherein said surface granule enzyme activity is detected using a FRET construct.

29. (Withdrawn) The method of Claim 26, wherein at least one of said five parameters is fluorescent dye release and wherein said fluorescent dye is a low pH concentration dye.

30. (Previously presented) The method of Claim 17, wherein the candidate bioactive agents are proteins or peptides.

31. (Previously presented) The method of Claim 17, wherein the candidate bioactive agents are small organic molecules.

32. (Previously presented) The method of Claim 17, further comprising comparing results obtained from said method to results obtained using a positive control, wherein the positive control is p21.

33. (Withdrawn) A method of screening for an alteration in cellular phenotype ,said method comprising:

a) combining a population of cells with a candidate bioactive agent;

b) sorting said population of cells based on at least five parameters using fluorescence activated cell sorting (FACS); and

c) detecting at least one cell of said population having said alteration in said cellular phenotype;

wherein said cellular phenotype is selected from a group of cellular phenotypes consisting of cell cycle, apoptosis, exocytosis, expression of a cell surface receptor, and expression of a reporter gent.

34. (Withdrawn) The method of Claim 31, wherein said cellular phenotype is exocytosis.

35. (Withdrawn) The method of Claim 32, wherein said sorting of said population of cells using fluorescence activated cell sorting (FACS) is based upon at least five parameters selected from the group consisting of: light scattering, fluorescent dye uptake, fluorescent dye release, annexin granule binding, surface granule enzyme activity, and the quantity of granule specific proteins.

36. (Withdrawn) The method of Claim 17, wherein said candidate bioactive agent is

obtained from a library of synthetic or natural compounds.

EVIDENCE APPENDIX

A Declaration under 35 U.S.C. § 1.131 (the "Fisher Declaration") is provided in this appendix.

COPY

DECLARATION OF JOSEPH FISHER UNDER 37 C.F.R. §1.131	Application Number	09/293,670
	Confirmation Number	5176
	Filing Date	April 16, 1999
	First Named Inventor	Joseph Fisher
	Examiner	Teresa Wessendorf
	Group Art	1639
	Attorney Docket No.	RIGL-036CIP

This Declaration with the attached Exhibits are being submitted in conjunction with the Applicants' Response to the Office Action dated February 24, 2006.

I, Joseph Fisher, M.D. Ph.D. do hereby declare as follows.

1. I am listed as an inventor of the above-referenced patent application.

2. Between June and September, 1997, I was a Scientist at Rigel Pharmaceuticals, Inc. (hereinafter "Rigel"). During this time, I was part of a program focused on the discovery of intracellularly-active peptides. The strategy employed by this program involved infecting cells with a library of retroviral vectors encoding candidate peptides, and selecting cells with an altered phenotype using fluorescence activated cell sorting (FACS)-based methods. The idea of using more than five FACS parameters to identify retrovirally-delivered, intracellularly-active peptides was developed before July 31, 1997.

3. I understand that the claimed subject matter of the above-referenced patent application relates to screening methods that include sorting a population of retrovirally infected cells using at least five fluorescence activated cell sorting (FACS) parameters. I

have been asked to provide factual evidence relating to my activities at Rigel with respect to the claimed subject matter before and after July 31, 1997.

4. Experiments confirming the applicability of FACS-based screening methods that employ at least five FACS parameters to the discovery of retrovirally-delivered intracellularly-active bioactive peptides were performed prior to July 31, 1997.

5. Exhibit A, which is a copy of pages 24 and 25 of my laboratory notebook, describes the results of an experiment in which cells were treated to induce exocytosis, and sorted using five FACS parameters. Exhibit A is dated prior to July 31, 1997. The top four graphs of page 25 show FACS results obtained from DMSO-treated cells (control), and the bottom four graphs of page 25 show FACS results obtained from A23187-treated cells (experimental). The top left graph of each group of four graphs shows results obtained from the parameter used to detect FM143, a fluorescent dye. The top right graph of each group of four graphs shows results obtained from the parameter used to detect FITC, another fluorescent dye. The bottom left graph shows results obtained from the parameter used to detect propidium iodide. The bottom right graph shows results obtained from parameter used to detect front light scatter as well as, independently, the parameter used to detect side light scatter. Thus, Exhibit A demonstrates the applicability of FACS methods that employ at least five FACS parameters to the discovery of retrovirally-delivered intracellularly-active bioactive peptides, before July 31, 1997.

6. Exhibit B, which is a copy of pages 112 to 120 of my laboratory notebook, describes an experiment in which and MC9 and CEM cells are transfected with a library of retroviral vectors that encode peptides. Exhibit B demonstrates that CEM and MC9 cells were transfected with a library of retroviral vectors between August 22 and August 27, 1997.

7. In September 1997, a method that included infecting cells with a library of retroviral vectors encoding candidate bioactive peptides, and selecting cells with an altered phenotype using five fluorescence FACS parameters was reduced to practice.

8. Exhibit C, which is a copy of pages 138 and 139 my laboratory notebook, describes an experiment in which retroviral vector library-infected cells are stimulated staurosporine to induce apoptosis, and sorted using five FACS parameters: side scatter ("ssc"), front light scatter ("fsc"), and three separate fluorescence parameters: ("fl1", "fl2" and "fl3"). Results for control cells not contacted with staurosporine are shown in the graphs on the left hand side of page 139, and results for experimental staurosporine-treated cells are shown in the graphs of the right hand side of page 139. Thus, Exhibit C demonstrates reduction to practice of a method that includes infecting cells with a library of retroviral vectors encoding candidate bioactive peptides, and selecting cells with an altered phenotype using five FACS parameters, on September 8, 1997.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

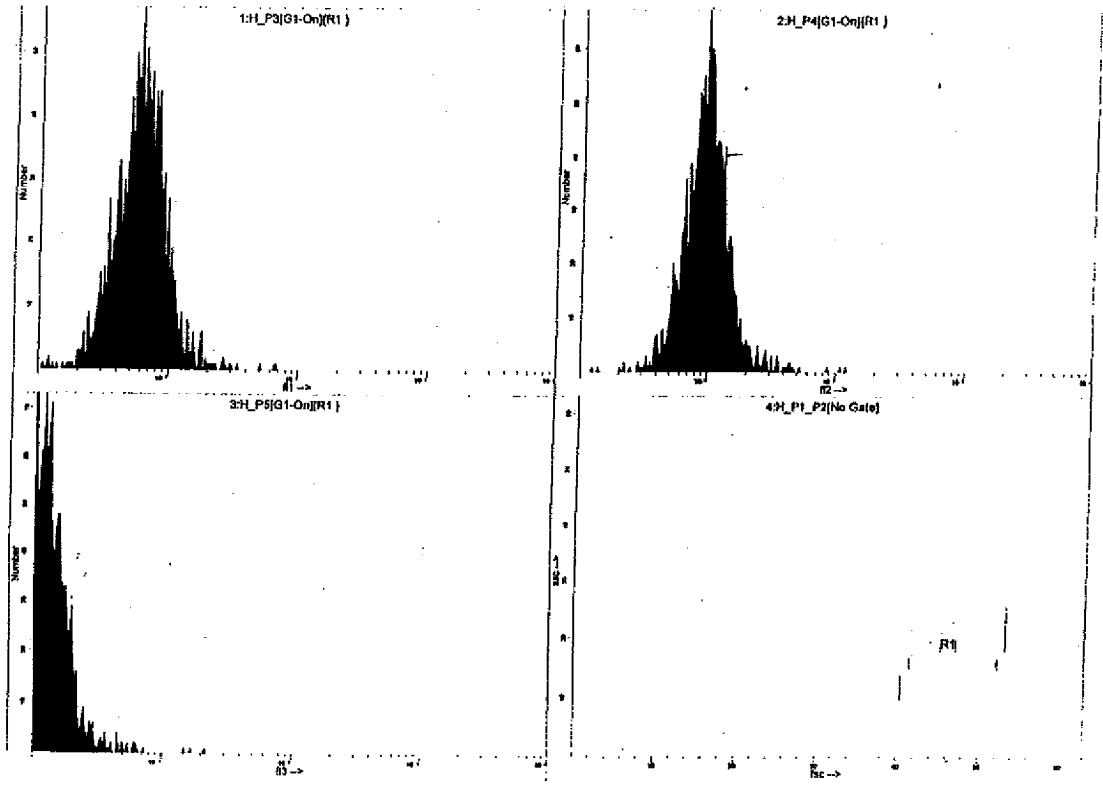
Respectfully submitted,

Date: June 25, 2006

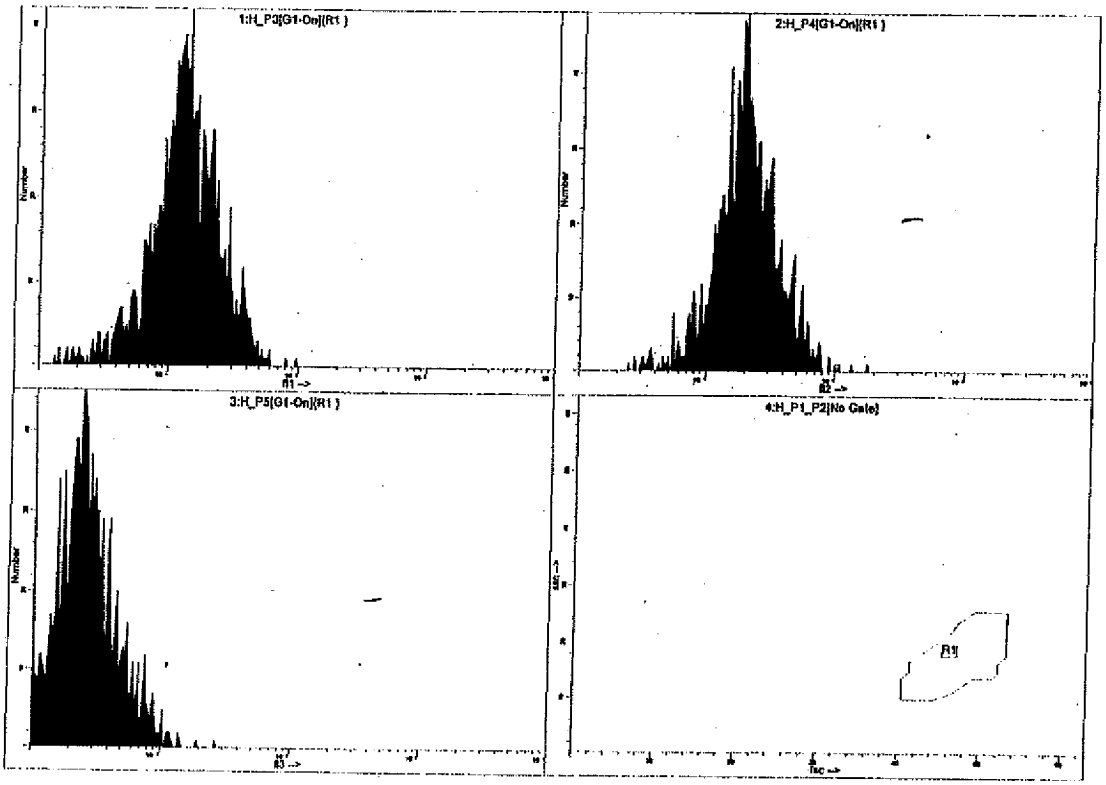
Joseph Fisher, M.D. Ph.D.,

Attachments: Exhibits A - C

EXHIBIT A



DMSO 10'
37°C



1ug/ml
A23187 10'
37°C

To Page No.

Witnessed & Understood by me,
James Jaen

Date

Invented by
Jack Fort

Recorded by

Date

Project No._____ EXHIBIT A

Book No._____ TITLE _____

From Page No.___

HMC-1 - Exocytosis Trace Dyes

- Try FM-143 and ConA FITC as exocytosis Tracers on HMC-1 Cells.
- HMC-1 Cells - From Alexis Spinner ~ 10^6 Cells/ml, Highly Viable
- Spin/Wash 5×10^6 Cells in MT
- Divide into 2 - ½ Incubate in MT 10' 37°C
  ½ " " " + Succinyl ConA 100ug/ml } 37°C 10'
- Wash SCA Cells 2x MT
- Take up Cells in 1ml MT (no BSA) in 4 Tubes
  A) DMSO } + FM-143 - 2.5 ug/ml ⇒ 37°C 10'
  B) + AZ3187 1ug/ml }
  C) DMSO } + Con-A-FITC 25 ug/ml ⇒ "
  D) AZ3187 " }

Wash Cells 1x in MT - Take up in 1ml MT for FACS

Saved Files as JMF012.001 > C
2 } D
3 > A
4 } B

To Page No.___

Witnessed & Understood by me,	Date	Invented by	Date

From Page No.___

8/22/97

Phoenix E Cell Transfection ⇒ For MC9 Cell Infection

- Use Susans Protocol (x2) So 2 wells of 6 well Plate / Transfection
- DNA- From Jenny Wang

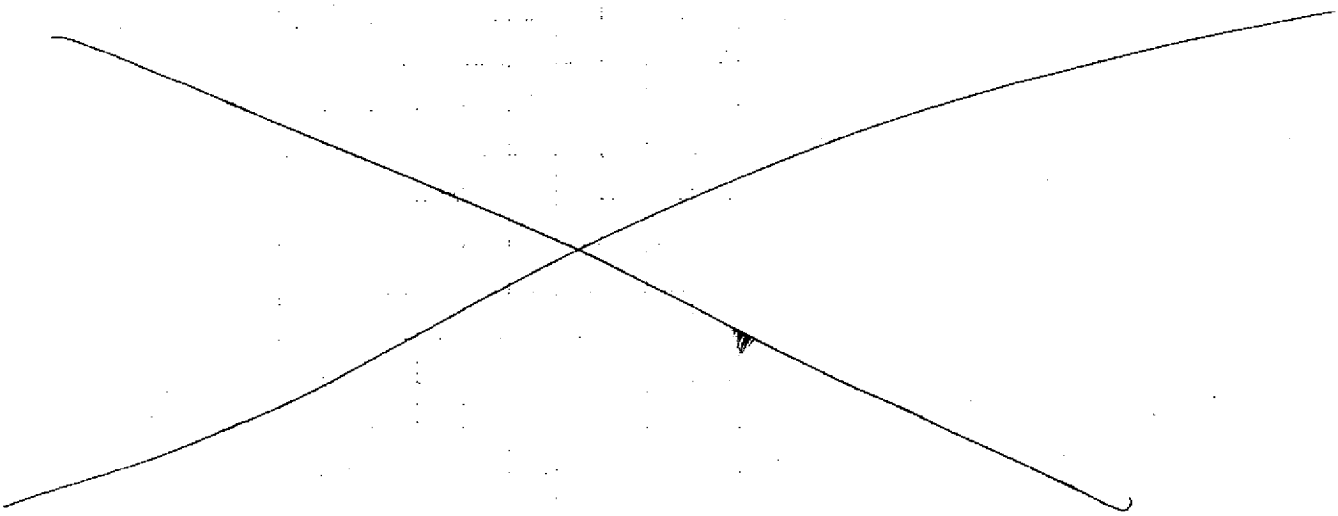
			Randy's Nomenclature	Jim's Nomenclature	
1	(10ug) =	6.6λ			Rab3a and Synaptotagmin
2		6.3λ			Constructs
3		8.9λ			
4		9.1λ			
5	- New IRes Hook		43-13	129.13	10ug = 11.6λ
6	- " " 6FP		010.25	010-25	10ug = 11.1λ

From Jim L

- Follow Susans Protocol- Put Precipitate / Chloroquine on cells at 11AM
- Nice "Peppery" Precipitate Seen on all Transfectats

Protocol on next Page.

7PM ⇒ Aspirate DNA
- Wash 1x in Phoenix Media
- Add 2ml/well Fresh Media



Witnessed & Understood by me,	Date	Invented by	Date
James Green	8/22/97	Joseph Fink	8/22/97

Protocol for transfection of Phoenix cells and infection of nonadherent target cells

Day 1:
seed Phoenix cells (Es or As) in 6 well plates at $8x10^5$cells in 1.5 ml (DMEM + 10% FBS + P/S) per well

Day 2: $CaPO_4$ Transfection

2wells

per well:

5ug DNA	10µg DNA
30.5ul 2M $CaCl_2$	61λ 2M CaCl2
219ul H_2O	438λ H_2O
250ul 2X HBS	500λ 2X HBS

allow all reagents to come to room temperature 30mins. before starting (do not warm up in H_2O bath)

add 50mM chloroquine at 2ul/well (50um final)

mix $CaPO_4$ reagents in 15ml polypropylene tube:
pipet 5ug DNA to side of tube
pipet 30.5ul of 2M $CaCL_2$ away from the DNA
mix the two together with the addition of 219ul of miliQ H_2O
then using a 1ml pipet, add 250ul of 2X HBS and quickly bubble air through the pipet for 2 to 10 secs. (the time is 2 HBS batch dependent)
immediately add mixture dropwise to well
microscopically visible precipitate should appear within a few minutes

incubate 8hrs
remove medium, wash once, and replace with 1.5ml medium

Day3:
move transfected plates to 32^0C

Day 4: Infection of target cells
collect virus supernatent from transfected wells (1.5 ml) into 15 ml tubes and add either 1.5ul of 5mg/ml polybrene or 1.5ul 5mg/ml protamine sulfate
cfg out cells and debris at 2500 RPM for 5 mins. or alternatively, filter through .45um acrodisc syringe filter
count target cells and distribute $5x10^5$ cells per virus supe to 15ml tubes and pellet 5 mins. 2500 RPM
resuspend each pellet of target cells with virus supe and transfer to one well of a 24 well plate
seal plate with parafilm and cfg at RT for 90 mins. at 2500 RPM
Remove parafilm and incubate plate over night at 32^0C

Day 5:
collect and pellet each well of target cells and resuspend in 4ml and transfer each to a 6cm plate

Day 7 or Day 8:
at 48 to 72 hrs. post infection target cells are ready to analyze for expression

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	8/22/97

From Page No.__

8/23/97

- Transfections of ϕE Cells - (Cont.)
- This morning - 24 hrs post Transfection Start
  Look at Cells by Fluorescence.
  GFP ⊕ Cells Seen in #3, 4, and 6
  3 and 4 must be CTI6 Vector (inducible with Ires GFP)
  1 and 2 " be neo Hook vector.
- Remove old Media
- Add 2ml/well of Warmed MC9 Media - 12AM

MC9 Positive Control Peptides

MC9 Cells - WT

Scaffold Hook	~75% Hook+	From Amy
Synaptotagmin	~50% "	
RAB	- " "	

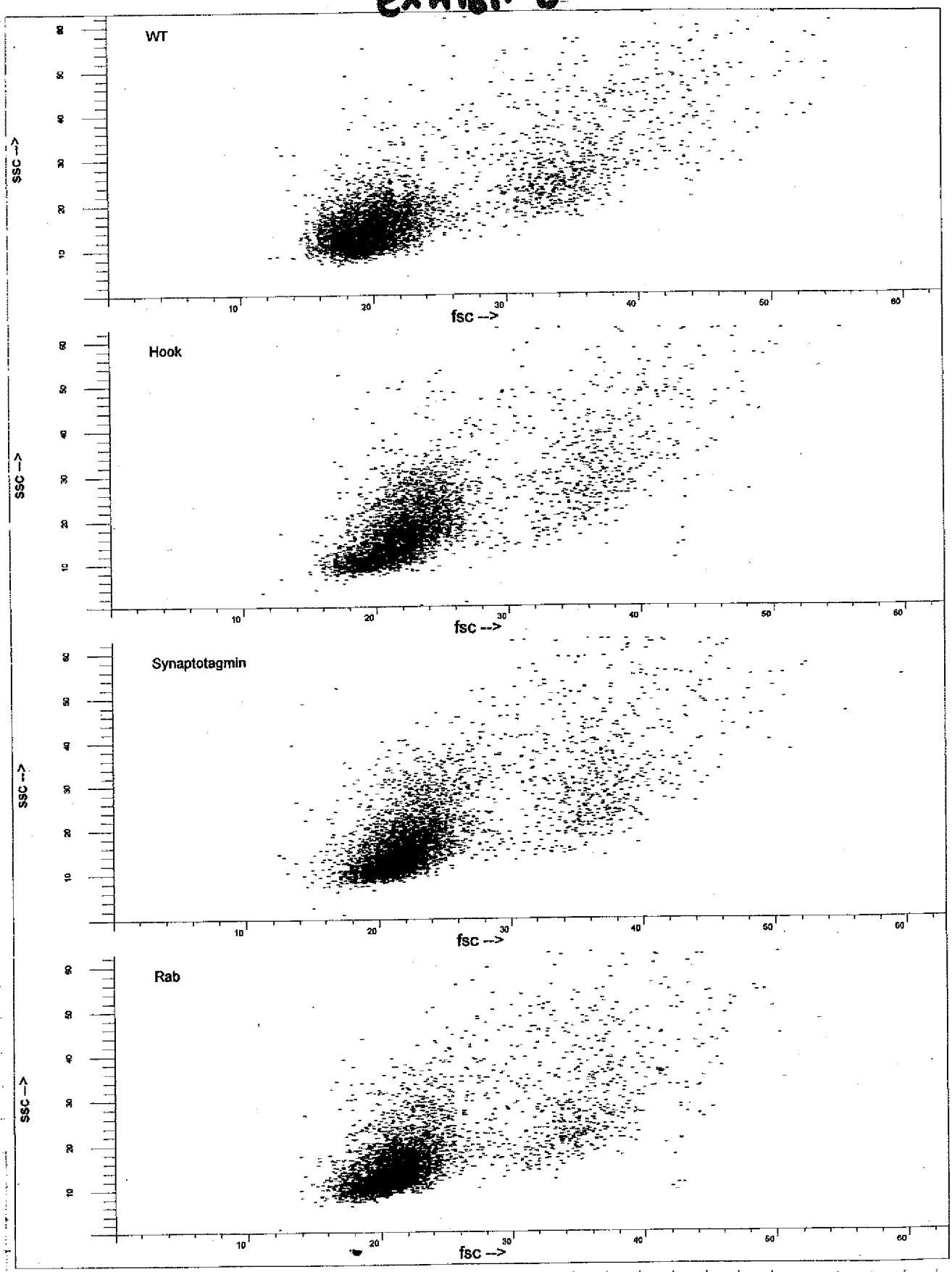
- Dsprate 2ml Cells, Take up in .3ml MT
  100l/Tube
  ⇒ one gets FM143 1um
  " " " " + 2um Ionomycin } 37°C ⇒ 30'
  " " PI

View in FACSCAN

- 001	WT	5 -	WT
- 2	Hook	6 +	"
- 3	Synaptotagmin	7 -	Hook
- 4	RAB	8 +	"
		9 -	Synaptotagmin
		10 +	"
		11 -	RAB
		12 +	"

To Page No.__

Witnessed & Understood by me,	Date	Invented by	Date
	8/26/97		8/23/97



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tnessed & Understood by me,

Date 8/26/04

Invented by

Recorded by

Date 8/28

Project No._____ EXHIBIT B

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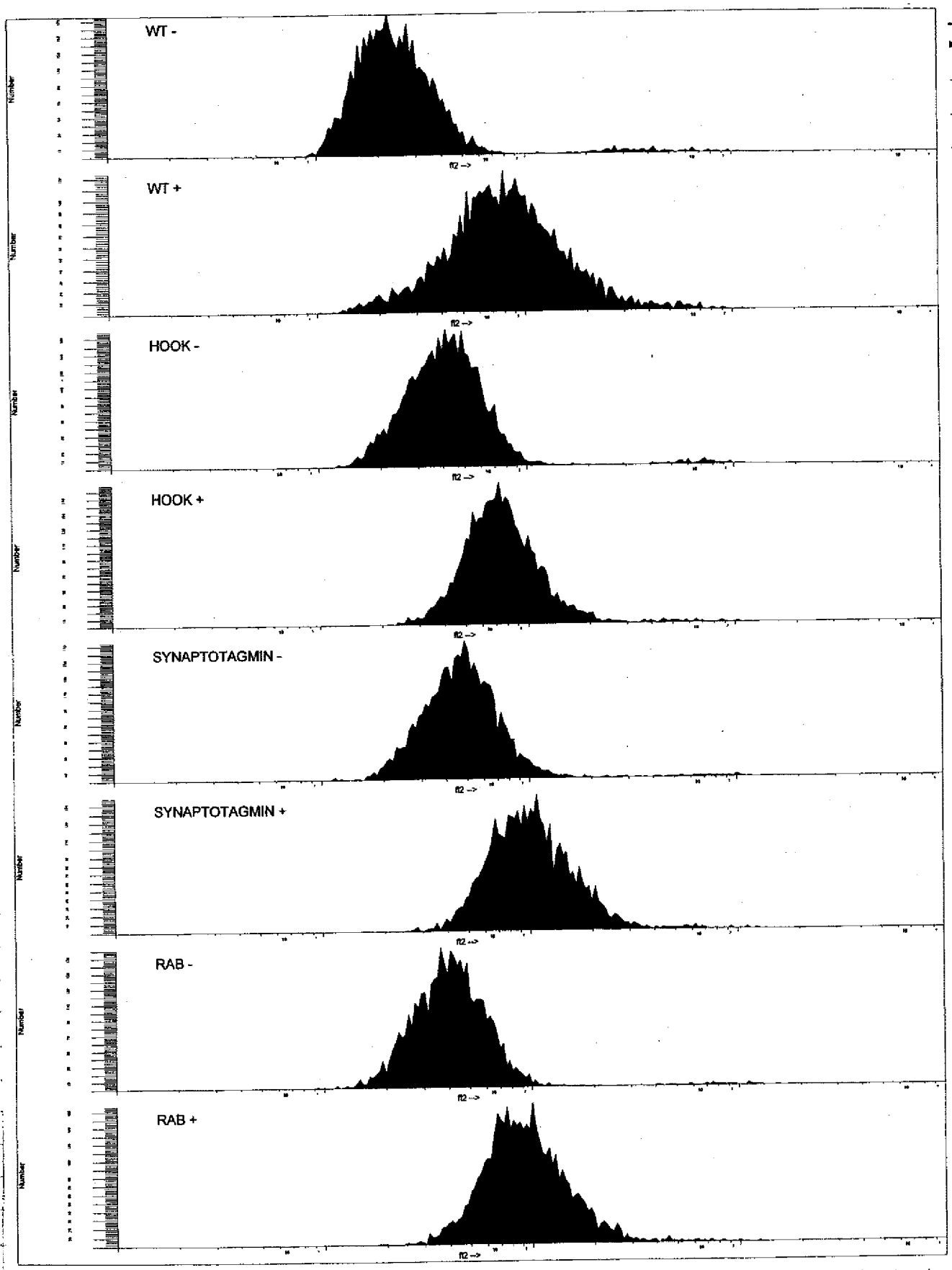
From Pag



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Witnessed & Understood by me,	Date	Invented by	Date
James Town	8/23/23	[illegible]	8/23

m Page No.

8/25

= MC9 Cell Infection (Cont)
- Wells 3/4 + 6 of Transfection Look Significantly Brighter for GFP Than Plate old on 8/23
- ~1PM - Remove Viral Supe - Spin at 2500 RPM x 15' RT
- MC9 Cells, ~2.5x10^6/ml
- Spin Down 2ml x 6 MC9 cells (~5x10^6/Tube)
- Add Viral Supes
- Divide Each into 2 Wells of a 6 Well Plate (~2ml / 2.5x10^6 Cells/well)
  Add 4λ of 5mg/ml Polyamine Sulfate / well so FC = 10μg/ml
- Seal Plates and Spin for 90' at 2500 RPM
- Culture ON at 37°C (~3:30 PM →)

- MC9 Cell Harvest - For Future cDNA Library Construction

Cells ~ 2x10^6/ml
- Spin down 200ml Cells
Wash 2x in Cold PBS / Aspirate
- Freeze on Dry Ice - 2 tubes x 2x10^6 Cells/Tube
- Store at -80°C

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		Recorded by	8/25/97

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8/26/97

MC9 Infections (Cont.)

2 x 6 well Plates infected Yesterday

- ~ 11AM, Take Cells out of Wells/Pool, Wash Cells with 2ml MC9 Media, Spin, Decant
- Take up Pellets 1→6 with 12ml MC9 Media and Plate in T-75's

→ Quick Look at #6 Showed some GFP ⊕ Cells.

ires GFP Library Inf. Transfection

- Susan Plated 20 60 mm Plates of ΦE Cells Yesterday, today ~ 40% Confluent
- Randy Supplied DNA 10-62 Library - 14mer, ires GFP 850 µg/ml
- For Each 60mm Plate add (Plates have 6ml of Media)

8λ Chloroquine (50mM)
10µg DNA (11.8λ)
122λ $CaCl_2$
876λ H_2O
1ml 2x HBS

Follow Standard Procedure

Transfected 17 Plates from 11:30AM → ~12:30AM

~ 6:30PM
- Aspirate Media
- Wash Cells 1x in PBS + Ca++
- Add Warm MC9 Media - 8ml/Plate Flask

→ 37°C ~ 7PM

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Witnessed & Understood by me.	Date	Invented by	Date
Jamie Tosen	8/26/97	Jon Fox	8/26/97

CEM - Library Infection

Library in pRESGFP, ~10^6 Complexity of random 14-mer Peptides - Part of 2nd IgE Screen Library.

- Yesterday Susan S. Put CEM Media on Library Infected ΦA Cells (After She Harvested her virus ~3PM) - Today Renae Spins (~4PM) Spin at 2500 RPM x 10' Add PS to 10μg/ml
- CEM Cells ~1.1×10^6/ml
  Spin Down 60ml (~ 6.6×10^7 Cells Total)
  - Divvi Pellets into 8 x 12ml Supers ⇒ / 8 T-75s ⇒ 8.25×10^6 cells/Flask
    Spin T-75s at 2500 RPM
    4:45 → 6:15
    Take out and Put at 37°C ON

Witnessed & Understood by me,	Date 8/26/97	Invented by	Date 8/26/97
		Recorded by	

Project No._____ EXHIBIT B

Book No._____ TITLE _____

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8/27/96

- ΦE Library Transfection

A few GFP⊕ cells seen today, but a minority

~4PM Transfer cells to 32°C

- Split MC9 cells for tomorrows Infection

CEM Cells - Library Infection

~1PM (22 hrs Post Infection Spin) Spin All infected CEM cells - Decant Supe

Take up in 90ml Fresh Media

Plate in 3x T-150's

- Take out 1ml of Library Infection, 1ml of WT Cells

Annexin - PE / PI Stain as done on 8/13 (use those Controls As well)

View in FACSCAN

Files 001 WT

002 Library Infected

⇒ See next page

Some GFP⊕ cells showing up in Library Infected after 22 hrs

Make MC9 Media

DMEM (has Pyruvate and Glutamine)

18mg/500ml Asparagine

1x Non-essential AA

0.05mM 2ME

Pen Strep 1x

10% HI FBS

10% T-Stim Conditioned Media

.2um Sterile Filter

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Witnessed & Understood by me, Date 10/24/97

Invented by [signature] Date 8/27/97

Recorded by

From Page No.___

9/8/97

CEM - Libray Infected - Apoptosis Induction

XX gave me Libray Infected Cells to test DNA Rescue Methods - CEM ~ 2.4×10^6/ml
Take 8ml (2×10^7 Cells) + 4ml Fresh Media, Bring to 1μM Staurasporine
→ 37°C 10AM → 4PM (6 Hours)
→ Annexin PE Stain as usual Procedure = files

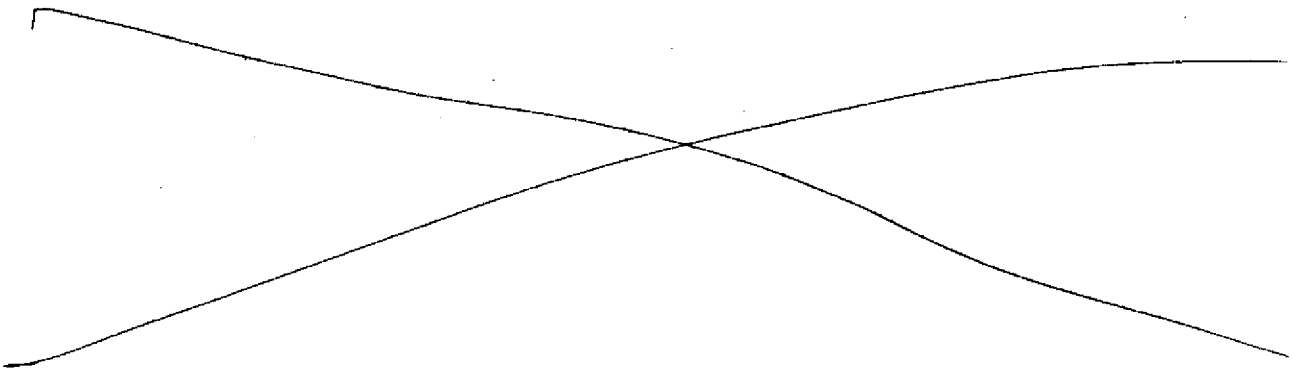
.015 6FP ONLY
16 Annexin PE ONLY
17 PI ONLY
18 6FP Libray - 0 Stavo
19 " " + Stavo 7hrs
} New Settings

CEM-Libray - Stavosporine treatment 2x (9/3) - Nov 5 days post treatment

Take .5ml of Culture - Add PI
- FACSCAN - .001 - Libray untreated
.002 Treated Stavo 2x

MC9 Libray - GFP Enriched

- GFP Enriched Cells from Last week - now ~ 2.8×10^6/ml x 100ml
- Split Back to ~ 10^6/ml for Tomorrow's Sort
- Remainder of Cells, ~ 2×10^8 Cells
Spin/Decant, Freeze in 5 vials (4×10^7/vial) at -80°C)



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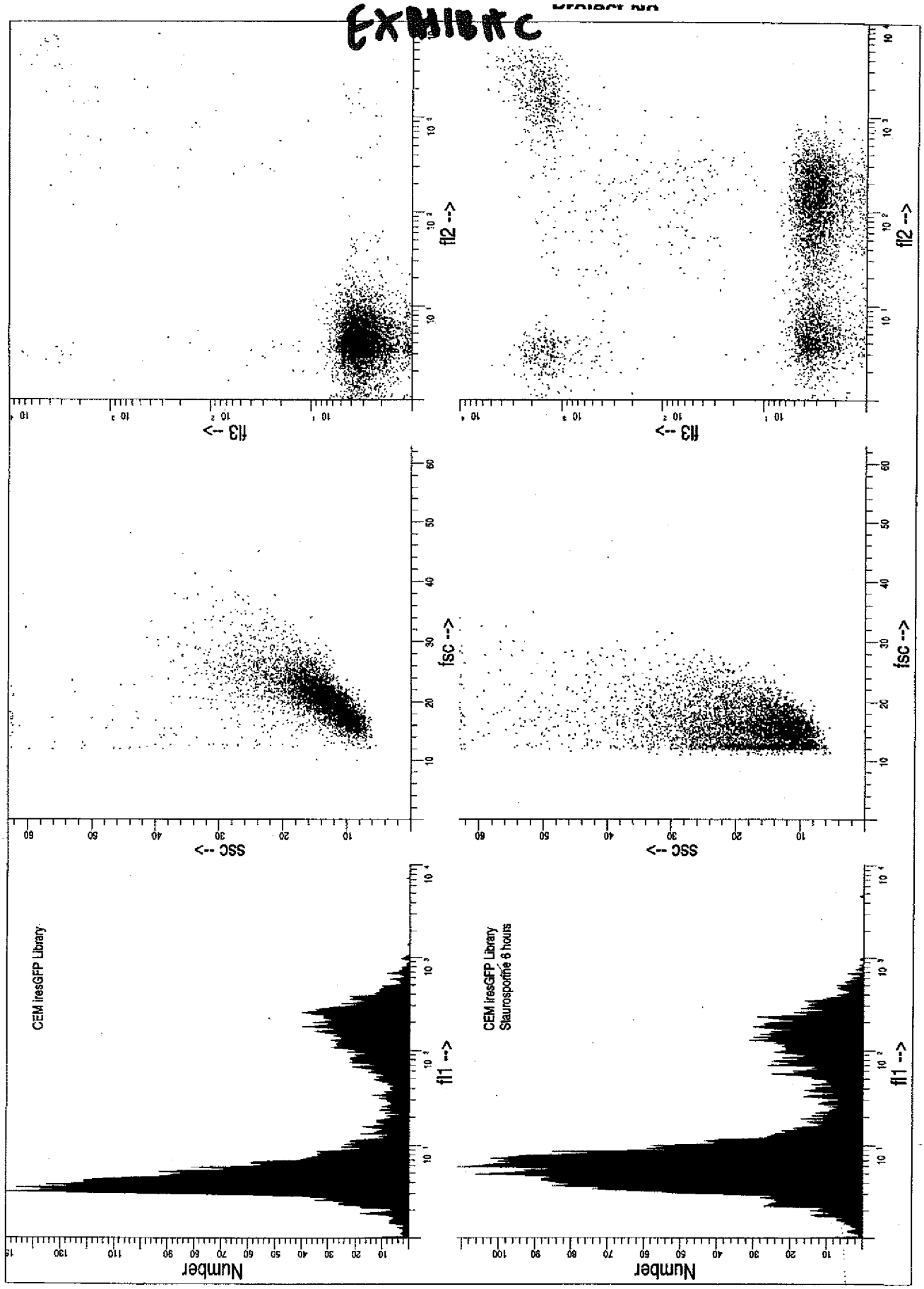
Witnessed & Understood By me	Date	Invented by	Date
[signature]	10/16/98	[signature]	9/8/97

EXHIBIT C



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d & Understood by me,

Date 10/26/99

Invented by

Recorded by

Date 9/8/97

RELATED PROCEEDINGS APPENDIX

As stated in the *Related Appeals and Interferences* section above, there are no other appeals or interferences known to Appellants, the undersigned Appellants' representative, or the assignee to whom the inventors assigned their rights in the instant case, which would directly affect or be directly affected by, or have a bearing on the Board's decision in the instant appeal. As such this section is left blank.